Original Article

Bacillus cereus as an Emerging Public Health Concern in Libya: Isolation and Antibiogram from Food of Animal Origin

Hesham T. Naas, Mohamed M. Zurghani, Aboubaker M. Garbaj, Salah M. Azwai[1], Hanan L. Eshamah, Fatim T. Gammoudi[1], Said K. Abolghait[2], Ashraf A. Moawad[3], Ilaria Barbieri[4], Ibrahim M. Eldaghayes[1]

Departments of Food Hygiene and Control and [1]Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Tripoli, Tripoli, Libya, [2]Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, [3]Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt, [4]Department of Genomics, The Lombardy and Emilia Romagna Experimental Zootechnic Institute (IZSLER), Brescia, Italy

Abstract

Background: This study was conducted to investigate the presence of *Bacillus cereus* in meat, meat products, and some seafood in Libya. **Materials and Methods:** One hundred and thirty-one samples were collected from different geographic localities in Libya. The samples were subjected to microbiological analysis for enumeration and isolation of *B. cereus* by conventional cultural, biochemical, and molecular identification using polymerase chain reaction (PCR) and partial sequencing of 16S rDNA techniques. **Results:** Of 131 samples, only 38 (29%) isolates were found to be *B. cereus* based on their cultural characteristics on Mannitol Egg-Yolk Polymyxin (MYP) medium that included 30% beef, 38.2% beef products (minced, burger, kabab, and sausage), 31.8% camel meat, and 48% chicken products (burger, sausage, kabab, and liver). However, *B. cereus* was not detected from mutton and seafood samples. Seventeen isolates were subjected to molecular identification using PCR and partial sequencing of 16S rDNA technique and confirmed to be *B. cereus*. The confirmed *B. cereus* strains were tested for their antibiotic sensitivity profiles and showed a high percentage of multiresistance phenotype. **Conclusions:** The results provide a better understanding of *B. cereus* isolated from food of animal origin in Libya and suggest that meat and meat products might play an important role in the spreading of *B. cereus* through the food chain with antimicrobial resistance characteristics.

Keywords: 16S rDNA, antibiogram, *Bacillus cereus,* Libya, meat products

Introduction

Bacillus cereus is a Gram-positive, rod-shaped, large size in single or short chains, facultative anaerobes, motile, β-hemolytic and mesophilic bacteria that produce heat-resistant endospores with a growth range of 10°C–48°C, with optimal growth at 28°C–35°C. In addition, it can grow in a broad pH range of 4.9–9.3.[1,2] It is a ubiquitous microorganism found in soils, water, dust, plants, animals, and humans. It is also isolated from contaminated foods of both plant and animal origins such as cereals, vegetables, milk and milk products, and meat and meat products causing foodborne illnesses in humans.[3] The occurrence of *B. cereus* as a meat contaminant was reported by some investigators, not only in raw meat but also in meat products.[4-6]

B. cereus has been isolated from the stools of 43% of healthy children and adults, at various concentrations.[7] *B. cereus* is an important foodborne pathogen, which causes two distinct types of food poisoning, i.e., diarrhea and emesis caused by two different types of toxins.[8,9] Three types of enterotoxins are associated with the diarrheal form of disease: three-component enterotoxin hemolysin BL, three-component nonhemolytic enterotoxin, and the single-component enterotoxin cytotoxin K. After consumption of contaminated food with *B. cereus,* the enterotoxins are released into the small intestine during vegetative growth following spore germination and by any surviving vegetative cells.[10] The diarrheal syndrome is caused by diarrheal toxins produced during the growth of the bacteria in the small intestine, while emetic syndrome is caused by emetic toxin produced by the bacteria during the growth phase in the food.[11] The incidence of the disease data related to

Access this article online

Quick Response Code:

Website: www.ljmsonline.com

DOI: 10.4103/LJMS.LJMS_5_18

Address for correspondence: Prof. Ibrahim M. Eldaghayes, Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Tripoli, P. O. Box 13662, Tripoli, Libya. E-mail: ibrahim.eldaghayes@vetmed.edu.ly

How to cite this article: Naas HT, Zurghani MM, Garbaj AM, Azwai SM, Eshamah HL, Gammoudi FT, *et al*. *Bacillus cereus* as an emerging public health concern in Libya: Isolation and antibiogram from food of animal origin. Libyan J Med Sci 2018;2:56-61.

B. cereus is extremely limited in Libya, because the disease associated with *B. cereus* may be underreported as very few of those affected seek medical attention owing to the mild nature and short duration of symptoms.[12] *B. cereus* was reported as a major causative agent of foodborne illness in the Netherlands in 2006 (causing 5.4% of the foodborne outbreaks) and in Norway in 2000 (causing 32% of foodborne outbreaks).[13] Scallan *et al*.[14] estimated that in the United States (US), *B. cereus* caused 0.7% of foodborne illness among 31 major pathogens. The Centers for Disease Control and Prevention reported on domestically acquired foodborne illness in the US that estimated number of episodes of *B. cereus* illness annually was given as 63,400 cases. Other outbreaks may go unreported or are misdiagnosed because of symptomatic similarities to *Staphylococcus aureus* intoxication (*B. cereus* vomiting type) or *Clostridium perfringens* food poisoning (*B. cereus* diarrheal type). The objectives of this study were to enumerate and isolate *B. cereus* from meat and meat products of different animal species and some seafood from different areas in Libya, to compare the cultural and molecular techniques as a tool for *B. cereus* confirmation, and to test the confirmed isolates for their antimicrobial susceptibility.

Materials and Methods

Collection and preparation of samples

A total of 131 samples [Table 1] including 49 raw meat samples of different species (beef, camel, chicken, and mutton), 59 meat products (beef products [minced, burger, kabab, and sausage] and chicken products [burger, kabab, sausage, and liver]), and 23 seafood (fish, clam, and shrimp), were collected from different cities in Libya (Tripoli, Regdalin, Janzour, and Tobruk). The samples were packed in sterile plastic bags, stored in an insulated icebox, and transferred to Food Hygiene and Control Laboratory Department, Faculty of Veterinary Medicine, University of Tripoli, on the same day of sample collection. All samples were subjected to *B. cereus* microbiological enumeration and isolation techniques followed by molecular identification by polymerase chain reaction (PCR) and partially sequencing of 16S rDNA. Decimal dilutions, culturing, and enumeration techniques of the samples were performed according to the methods described by the American Public Health Association.[15] Briefly, 25 g from each sample was aseptically transferred into a sterile stomacher bag (Seward Medicals, UK) and homogenized (Stomacher 400, Seaward Medicals, UK) with 225 mL of sterile 0.1% (w/v) peptone water (Park Scientific, UK) at 230 rpm for 2 min.

Table 1: *Bacillus cereus* in meat, meat products, and seafood samples (CFU/g)

Type of samples	Number of samples	Mean	Minimum	Maximum
Beef				
Meat	10	8×10^3	7×10^3	9×10^3
Minced	11	1.2×10^3	9×10^2	1.4×10^3
Burger	12	7.6×10^3	5.5×10^3	9.7×10^3
Kabab	5	2×10^4	1.3×10^4	2.7×10^4
Sausage	6	1.2×10^3	9.8×10^2	1.4×10^3
Chicken				
Meat	9	1.2×10^3	9.4×10^2	1.5×10^3
Liver	5	1×10^3	9.8×10^2	1.1×10^3
Kabab	5	1.9×10^4	1.6×10^4	2.1×10^4
Burger	10	8.7×10^3	6.4×10^2	1.1×10^4
Sausage	5	1.2×10^3	1×10^3	1.4×10^3
Camel meat	22	4.4×10^4	3.5×10^3	8.5×10^4
Mutton	8	0	0	0
Seafood	23	0	0	0
Total	131			

Enumeration and isolation of *Bacillus cereus*

Enumeration and isolation of *B. cereus* were performed using *B. cereus* selective differential MYP Agar Base (Park Scientific ltd. Co., UK).[16] MYP plates were surface plated by spreading of 0.1 mL of appropriate tissue homogenate serial dilutions and then incubated at 37°C for 24 h. MYP plates were examined for the presence of colonies surrounded by precipitate zone, which indicates that lecithinase is produced. *B. cereus* colonies are usually a pink color surrounded by precipitate zone with the same color [Figure 1]. Countable plates were those containing 15–150 colonies.[17]

Identification of *Bacillus cereus* by polymerase chain reaction and partial sequencing of 16S rDNA

Seventeen randomly selected positive *B. cereus* isolates on MYP medium were sent for sequencing of partial amplification 16S rDNA (464 bp) of isolated *B. cereus* strains using the universal oligonucleotide primers.

DNA extraction and amplification of 16S rDNA

DNA extraction of *B. cereus* isolates was performed by GF-1 bacterial DNA extraction kit (Cat. # GF-BA-100, Vivantis, Malaysia) as described in a previous study.[18] The 16S rDNA was amplified using the universal oligonucleotide primers; forward: S-D-Bact-0341-b-S-17 5′-CCTACGGGNGGCWGCAG-3′ and Reverse: S-D-Bact-0785-a-A-21 5′-GACTACHVGG GTATCTAATCC-3′.[19]

Electrophoresis, gel extraction, and DNA sequencing

The amplified 16S rDNA PCR fragment (464 bp) was excised from the gel, and the DNA was purified using GF-1

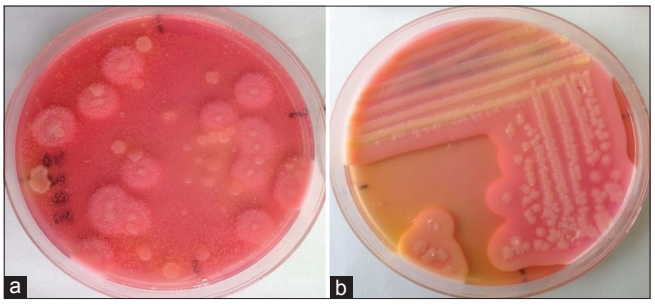

Figure 1: (a and b) Typical colonies of *Bacillus cereus* grown on Mannitol Egg-Yolk Polymyxin agar plate (pink colonies surrounded by a zone of precipitation [lecithinase-positive], after overnight incubation at 37°C])

Ambi Clean kit (Cat. # GF-GC-100, Vivantis, Malaysia) as described previously.[18] The purified 16S rDNA amplicons underwent cycle sequencing with BigDye® Terminator v1.1 kit (AB Applied Bioscience, TECHNE, TC-512, USA) and were sequenced on four capillary ABI PRISM® 3130-Avant Genetic Analyzer at IZSLER Istituto Zooprofilattico Sperimentale Della Lombardia e dell'Emilia Romagna, Italy. Sequences were assembled and edited using the SeqMan module within Lasergene package (DNA Star Inc., Madison, WI, USA). The obtained consensus sequences were subjected to BLAST search both at NCBI (http://www.ncbi.nlm.nih.gov/pubmed) and at 16S bacterial cultures Blast Server for the identification of prokaryotes (http://bioinfo.unice.fr/blast/).

Biochemical identification of isolated strains

The identified *B. cereus* strains by PCR technique were examined for their typical biochemical reactions (hemolysin, lecithinase, sugar fermentation: arabinose, mannitol, and xylose).[20]

Strains preparation for antibiogram

On confirmation by PCR and partial sequencing of 16S rDNA gene, isolated strains of *B. cereus* were preserved by freezing at −80°C in vials containing brain–heart Infusion broth (BHI, Oxoid, UK) supplemented with 30% (v/v) glycerol (DBH, UK). To propagate the frozen culture, vial was thawed at room temperature, and 0.5 mL of thawed culture was transferred to 5 mL of BHI broth and incubated for 24 h at 37°C. The inoculum was prepared from the second transfer of that culture (0.5 mL) to another 5 mL of BHI broth then incubated for 16–18 h at 37°C.

Antibiogram assay

After the overnight incubation of tested strain, Mueller Hinton agar plates (Oxoid, UK) were surface swabbed.[21] The selection of antibiotics (24) was based on their common use in human and animals which included amoxycillin (10 µg), amoxycillin/clavulanic acid (30 µg), ampicillin (10 µg), bacitracin (10 µg), penicillin G (10 µg), methicillin (5 µg), erythromycin (15 µg), gentamicin (10 µg), kanamycin (30 µg), lincomycin (10 µg), tobramycin (10 µg), vancomycin (10 µg), levofloxacin (5 µg), clindamycin (2 µg), cefotaxime (30 µg), doxycycline (30 µg), ciprofloxacin (5 µg), cloxacillin (5 µg), nitrofurantoin (300 µg), oxytetracycline (30 µg), streptomycin (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), and sulfamethoxazole/trimethoprim (25 µg). All antibiotics used were obtained from Oxoid, except gentamicin (10 µg) and methicillin (5 µg) which were obtained from Bioanalyse, Turkey. Under aseptic condition, antibiotic discs were dispensed and lightly pressed onto the agar surface and then incubated overnight at 37°C. The bacterial growth around each disc was observed. The clear zones around antibiotic discs that have no growth, referred to as the zone of inhibition, were measured and scored as sensitive, intermediate (reduced susceptibility), or resistant according to the CLSI 2014 guidelines.[22] The antibiotic resistance index (ARI) and multiple antibiotic resistance (MAR) index for all the isolated bacteria were calculated as follows:[23,24]

$$\text{ARI} = \frac{\text{Number of antibiotic resistant bacterial isolates}}{\text{Total number of test bacterial isolates} \times \text{Number of antibiotic tested}}$$

and

$$\text{MAR index} = \frac{\text{Number of antibiotics to which the isolate showed resistance}}{\text{Number of total antibiotics exposed to the isolate}}$$

and interpreted according to Krumperman.[24] MAR index ≤0.2 was considered low risk and ≥0.2 was considered as high risk.

Results

Enumeration and isolation of *Bacillus cereus*

One hundred and thirty-one samples from various regions of Libya comprising raw meat (49), meat products of different species (59), and seafood (23) were tested for the presence of *B. cereus* using MYP medium [Figure 1]. *B. cereus* was isolated from 41 samples of raw meat (beef, chicken, and camel meat) – 30%, 33.3%, and 31.8% with mean counts 8×10^3, 1.2×10^3, and 4.4×10^4 CFU/g, respectively. *B. cereus* was not isolated from mutton and seafood samples. For meat products (59), isolation rate of *B. cereus* on MYP agar plates of beef product samples was 38.2% with counts ranging from 9×10^2 to 2.7×10^4 CFU/g and of chicken meat products was 48% with counts ranging from 6.4×10^2 to 2.1×10^4 CFU/g [Tables 1 and 2]. The occurrence of *B. cereus* was 25% in beef burger with mean count of 7.6×10^3 CFU/g. Meanwhile, in beef kabab, the isolation rate was 80% [Table 2] with mean count of 2×10^4 CFU/g. Detection of *B. cereus* in chicken burger was 30% with mean count of 8.7×10^3 CFU/g. However, in chicken kabab, the incidence rate was 60% with mean count of 1.9×10^4 CFU/g.

Identification of *Bacillus cereus*

Of 38 suspected *B. cereus* isolates from MYP plates, 17 (45%) were randomly selected [Table 2] and sent for partial sequencing of 16S rDNA (464 bp) of *B. cereus* using the universal oligonucleotide primers [Figure 2]. All seventeen suspected isolates were confirmed to be *B. cereus* by partial sequencing of 16S rDNA technique.

Antibiotic resistance phenotype

All confirmed *B. cereus* isolates showed resistance to amoxycillin, amoxicillin/clavulanic acid, ampicillin, bacitracin, penicillin G, cefotaxime, and cloxacillin, while 94% of isolates were resistant to lincomycin and 88% to methicillin and sulfamethoxazole/trimethoprim. However, more than 80% of the isolates were sensitive to gentamicin, tobramycin, vancomycin, ciprofloxacin, nitrofurantoin, tetracycline, and chloramphenicol. Levofloxacin and doxycycline were effective against all tasted isolates. The calculated ARI for the tested isolates was 0.03, while the MAR indicates MDR [Table 3].

Table 2: Prevalence of suspected *Bacillus cereus* in meat, meat products, and seafood samples

Type of sample	Number of samples	Number of suspected *B. cereus* grew on MYPA (%)	Number of sequenced *B. cereus* isolates	Number of positive *B. cereus* by 16S rDNA sequencing (%)
Beef				
Meat	10	3 (30)	1	1 (100)
Minced	11	3 (27)	1	1 (100)
Burger	12	3 (25)	1	1 (100)
Kabab	5	4 (80)	2	2 (100)
Sausage	6	3 (50)	1	1 (100)
Chicken				
Meat	9	3 (33.3)	1	1 (100)
Liver	5	3 (60)	1	1 (100)
Kabab	5	3 (60)	2	2 (100)
Burger	10	3 (30)	2	2 (100)
Sausage	5	3 (60)	1	1 (100)
Camel meat	22	7 (31.8)	4	4 (100)
Mutton	8	None	None	None
Seafood	23	None	None	None
Total (%)	131	38 (29)	17 (45)	17 (100)

MYPA: Mannitol Egg-Yolk Polymyxin Agar, *B. cereus*: *Bacillus cereus*

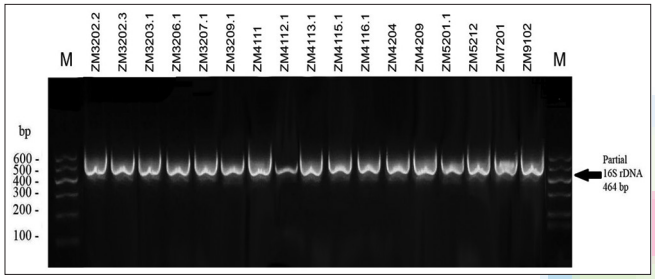

Figure 2: Representative gel of partial amplification of 16S rDNA (464 bp) products of isolated *Bacillus cereus* strains using the universal oligonucleotide primers. First and last lanes contain DNA marker

Discussion

Fresh raw meat and meat products are susceptible to biochemical changes due to the microbial growth at ambient temperatures 20ºC–35ºC, which are high in some countries (Libya). The meat products should be kept at low temperature (refrigerating or freezing) to extend their shelf life.[25]

In general, the incidence of *B. cereus* all over the collected samples of beef and beef products was 36.4% (16/44) [Table 2]. The results of this study are similar to the results obtained by Tewari[26] (27.8%). Whereas the results of other studies were very low compared to the results of this study as that with Konuma[27] (6.6%) and Perera and Ranasinghe[28] (1%). These differences may be due to the exposure to many sources of contamination during slaughtering, dressing and storage process. Currently, no data have been published on the incidence of *B. cereus* in camel meat as recorded in this study. The incidence of *B. cereus* in camel meat was 31.8% (7/22) with count ranged from 3.5×10^3 to 8.5×10^4 with mean value of $4.4 \times 10^4 \pm 4 \times 10^4$ CFU/g. This study failed to detect *B. cereus* strains from mutton samples that may be due to low bacterial load. Meanwhile, Akhlaq[29] isolated *B. cereus* from both goat and mutton samples and noted that the level of *B. cereus* was highest among all species of studied bacteria and significantly greater than the other pathogenic strains found in mutton and goat meat samples.

The incidence of *B. cereus* in beef kabab and minced beef was less than the results obtained by Fang *et al*.[30] Smykal and Rokoszewska[31] indicated that over a 7-year period (1964–1971), the prevalence of *B. cereus* was 13.3% in meat and meat products. The incidence and count of *B. cereus* in the beef sausage samples were in agreement with the data reported by Nortjé *et al*.[32] The results recorded by Eglezos *et al*.[12] for cooked sausage rolls were lower compared with the results obtained from this study. However, it has been proved that the occurrence rate of *B. cereus* is often much higher in raw or undercooked products compared with cooked ones because of the absence of heating process in order to reduction of microbial load.[33] These differences as a high incidence in the current investigation in red meat products may also be due to cross-contamination during production and preparation of meat or due to type of preparation of the product, defects in hygienic measures, or kind of additives and spices.[20]

In general, the incidence of *B. cereus* in chicken meat samples was lower than the results recorded by Sharma *et al*.,[34] and greater than the results reported by Nortjé *et al*.[32] The results in this study were similar with the results reported by Perera and Ranasinghe.[28] Furthermore, the incidence of *B. cereus* in chicken products (kabab, sausage, and liver) was high compared to the results reported by Raja *et al*.,[35] whereas the results reported by Sooltan *et al*.[36] were lower than that of the current study. On the other hand, the results recorded by Abostate *et al*.[37] were similar to the results of this study, except in minced chicken. The results of this study were lower than that found by Smith *et al*.[38] These variations in the results

Table 3: Antibiogram Results for 17 *Bacillus cereus* isolated form meat and meat products

Number	Antimicrobial agent	Resistant (%)	Sensitive (%)	Intermediate (%)
1	Amoxycillin 10 µg	100	0	0
2	Amoxycillin/clavulanic acid 30 µg	100	0	0
3	Ampicillin 10 µg	100	0	0
4	Bacitracin 10 µg	100	0	0
5	Penicillin G 10 µg	100	0	0
6	Methicillin 5 µg	88.2	5.9	5.9
7	Erythromycin 15 µg	0	17.7	82.4
8	Gentamicin 10 µg	0	94.1	5.9
9	Kanamycin 30 µg	5.9	52.9	41.2
10	Lincomycin 10 µg	94.1	0	5.9
11	Tobramycin 10 µg	5.9	82.4	11.7
12	Vancomycin 10µg	0	82.4	17.7
13	Levofloxacin 5 µg	0	100	0
14	Clindamycin	5.9	11.8	82.4
15	Cefotaxime 30 µg	100	0	0
16	Doxycycline 30 µg	0	100	0
17	Ciprofloxacin 5 µg	0	94.1	5.9
18	Cloxacillin 5 µg	100	0	0
19	Nitrofurantoin 300 µg	0	82.4	17.7
20	Oxytetracycline 30 µg	17.7	5.9	76.5
21	Streptomycin 10 µg	0	41.2	58.8
22	Tetracycline 30 µg	5.9	88.2	5.9
23	Chloramphenicol 30 µg	0	94.1	5.9
24	Sulfamethoxazole/trimethoprim 25 µg	88.2	5.9	5.9

were attributed to the quality of raw materials and the hygienic state during preparation and processing of the product. The presence of *B. cereus* in processed poultry products is due to surviving of spores from raw poultry and added ingredients and contamination with either spores or cells during processing.[20]

All of 17 randomly selected positive *B. cereus* isolates on MYP medium that sent for sequencing of partial amplification 16S rDNA (464 bp) of isolated *B. cereus* strains using the universal oligonucleotide primers were identified as *B. cereus* (100%) by PCR technique. These results showed that MYP media could be used as selective and differential media for *B. cereus* because no other microorganism could grow on it. In another study, among the 150 food samples (raw meats and meat products) analyzed, 40% (60) were positive for isolation and 39.33% (59) turned out positive by direct PCR (targeting sequence within *gyrB* gene).[39]

Regarding antibiotic susceptibility, all isolated strains of *B. cereus* in this study from meat and meat products were resistant to 7 out of 24 (29%) antibiotics and β-lactam antibiotics were not effective in all *B. cereus* isolates similar to other published data.[34,40] Of 24 antibiotics, 2 (8.3%) were highly effective against all isolates characterized in this study (levofloxacin and doxycycline); this finding was lower than the sensitivity percentage of doxycycline 88.23% reported by Luna *et al*.,[41] but levofloxacin sensitivity was similar. The current results showed that the β-lactam antibiotics were not effective in all *B. cereus* isolates as a consequence of *β-lactamase* action which secreted by *B. cereus* also in the presence of clavulanic acid, but amoxycillin/clavulanic acid was highly sensitive as confirmed by Pirzada *et al*.[42] The results obtained from this study showed multidrug resistance of *B. cereus* isolates according to the data published by Florişteanu *et al*.[20] Variations in the percentages of susceptibility to antibiotics may be due to the differences in the concentrations of antibiotic agents, differences in the sources of isolates, drug resistance transfer, and the widespread misuse of the antibiotics in the field.[43]

Conclusions

Our results showed that the isolation of *B. cereus* from Libyan meat and meat products of different species and some seafood represents an important aspect for food safety. The results provide a better understanding of *B. cereus* isolated from food of animal origin in Libya and suggest that meat and meat products might play an important role in the spreading of *B. cereus* through the food chain with antimicrobial resistance characteristics. This high incidence in meat products could be attributed to low hygienic practices, contaminated additives, and cross-contamination during the preparation of such products. Thus, hygienic slaughter of animals in slaughterhouses could improve the safety of carcasses and raw meat used in meat product formulation.

Financial support and sponsorship

This research was part of a project titled "Genetic authentication of bacterial isolates from meat and milk products in Libya and establishing the FLBC" that was supported by a grant

provided by Authority of Natural Science Research and Technology (Former name: The Libyan Authority for Research, Science and Technology).

Conflicts of interest

There are no conflicts of interest.

References

1. Duport C, Jobin M, Schmitt P. Adaptation in Bacillus cereus: From Stress to Disease. Front. Microbiol 2016;7:1550. Doi: 10.3389/fmicb.2016.01550.
2. Schneider KR, Parish ME, Goodrich RM, Cookingham T. Preventing Foodborne Illness: *Bacillus cereus* and *Bacillus anthracis*. Gainesville: Florida Cooperative Extension Service, University of Florida; 2004.
3. Larsen B, Ekelund S, Jørgensen L, Bremmelgaard A. Determination of the exocrine pancreatic function with the NBT-PABA test using a novel dual isotope technique and gas chromatography-mass spectrometry. Scand J Clin Lab Invest 1997;57:159-65.
4. Rather MA, Aulakh RS, Gill JP, Ghatak S. Enterotoxin gene profile and antibiogram of *Bacillus cereus* strains isolated from raw meats and meat products. J Food Saf 2012;32:22-8.
5. Stenfors Arnesen LP, Fagerlund A, Granum PE. From soil to gut: *Bacillus cereus* and its food poisoning toxins. FEMS Microbiol Rev 2008;32:579-606.
6. Soleimani M, Hosseini H, Neyestani Z, Siadati S, Pilevar Z. Occurrence of *Bacillus cereus* in beef burger marketed in Tehran, capital of Iran. J Food Q Hazards Cont 2017;4:70-3.
7. Humphries RM, Linscott AJ. Laboratory diagnosis of bacterial gastroenteritis. Clin Microbiol Rev 2015;28:3-1.
8. Dierick K, Van Coillie E, Swiecicka I, Meyfroidt G, Devlieger H, Meulemans A, *et al.* Fatal family outbreak of *Bacillus cereus*-associated food poisoning. J Clin Microbiol 2005;43:4277-9.
9. Schoeni JL, Wong AC. *Bacillus cereus* food poisoning and its toxins. J Food Prot 2005;68:636-48.
10. Wijnands LM, Pielaat A, Dufrenne JB, Zwietering MH, van Leusden FM. Modelling the number of viable vegetative cells of *Bacillus cereus* passing through the stomach. J Appl Microbiol 2009;106:258-67.
11. Ehling-Schulz M, Fricker M, Grallert H, Rieck P, Wagner M, Scherer S, *et al.* Cereulide synthetase gene cluster from emetic *Bacillus cereus*: Structure and location on a mega virulence plasmid related to bacillus anthracis toxin plasmid pXO1. BMC Microbiol 2006;6:20.
12. Eglezos S, Huang B, Dykes GA, Fegan N. The prevalence and concentration of *Bacillus cereus* in retail food products in Brisbane, Australia. Foodborne Pathog Dis 2010;7:867-70.
13. Wijnands LM. *Bacillus cereus* Associated Food Borne Disease Quantitative Aspects of Exposure Assessment and Hazard Characterization. Thesis Wageningen University, Wageningen, The Netherlands; 2008.
14. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, *et al.* Foodborne illness acquired in the United States – Major pathogens. Emerg Infect Dis 2011;17:7-15.
15. Downes FP, Ito K, Association APH. Compendium of Methods for the Microbiological Examination of Foods. 4th Ed. American Public Health Association, USA. 2001.
16. Mossel DA, Koopman MJ, Jongerius E. Enumeration of *Bacillus cereus* in foods. Appl Microbiol 1967;15:650-3.
17. Tallent SM, Rhodehamel EJ, Harmon SM, Bennett RW. (2012). Bacillus cereus. In Bacteriological analytical manual. U.S. Food and Drug Administration, Washington, DC., ed. (Online) Silver Spring, 2012. Available from: http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070875.htm. [Lat accessed on 2017 Oct 18].
18. Azwai SM, Alfallani EA, Abolghait SK, Garbaj AM, Naas HT, Moawad AA, *et al.* Isolation and molecular identification of *Vibrio* spp. by sequencing of 16S rDNA from seafood, meat and meat products in libya. Open Vet J 2016;6:36-43.
19. Herlemann DP, Labrenz M, Jürgens K, Bertilsson S, Waniek JJ, Andersson AF, *et al.* Transitions in bacterial communities along the 2000 km salinity gradient of the baltic sea. ISME J 2011;5:1571-9.
20. Floriştean V, CreŃu C, Carp-Cărare M. Bacteriological characteristics of *Bacillus cereus* isolates from poultry. Bull USAMV CN 2007;64:1-2.
21. Coyle MB. Manual of Antimicrobial Susceptibility Testing. Washington, D.C: American Society for Microbiology Press; 2005. p. 25-39.
22. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement M100 S24. Wayne (PA): Clinical and Laboratory Standards Institute; 2014.
23. Al-Mujaini A, Al-Kharusi N, Thakral A, Wali UK. Bacterial keratitis: Perspective on epidemiology, clinico-pathogenesis, diagnosis and treatment. Sultan Qaboos Univ Med J 2009;9:184-95.
24. Krumperman PH. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. Appl Environ Microbiol 1983;46:165-70.
25. Kalalou I, Faid M, Touhami Ahami A. Extending shelf life of fresh minced camel meat at ambient temperature by *Lactobacillus dlbrueckii* subsp. delbrueckii. Electron J Biotechnol 2004;7:5-6.
26. Tewari A, Singh SP, Singh R. Incidence and enterotoxigenic profile of *Bacillus cereus* in meat and meat products of Uttarakhand, India. J Food Sci Technol 2015;52:1796-801.
27. Konuma H, Shinagawa K, Tokumaru M, Onoue Y, Konno S, Fujino N, *et al*. Occurrence of *Bacillus cereus* in meat products, raw meat and meat product additives. J Food Prot 1988;51:324-6.
28. Perera ML, Ranasinghe GR. Prevalence of *Bacillus cereus* and associated risk factors in chinese-style fried rice available in the city of Colombo, Sri Lanka. Foodborne Pathog Dis 2012;9:125-31.
29. Akhlaq M. Evaluation of microbiological status of sheep and goat meat available within the city area of Dera Ismail Khan, Pakistan. J Cell Anim Therap 2015;1:1-7.
30. Fang TJ, Wei QK, Liao CW, Hung MJ, Wang TH. Microbiological quality of 18 degrees C ready-to-eat food products sold in Taiwan. Int J Food Microbiol 2003;80:241-50.
31. Smykal B, Rokoszewska J. *Bacillus cereus* as an etiological factor in food poisoning. Rocz Panstw Zakl Hig 1976;27:47-53.
32. Nortjé G, Vorster S, Greebe R, Steyn P. Occurrence of *Bacillus cereus* and *Yersinia enterocolitica* in South African retail meats. Food Microbiol 1999;16:213-7.
33. Jay JM, Loessner MJ, Golden DA. Modern Food Microbiology. 7th ed. New York: Springer Science; 2005.
34. Sharma C, Sharma D, Gill J, Aulakh R, Sharma J. *Bacillus cereus* from the food of animal origin in India and its public health significance. Acta Vet Scand 2003;44:1-2.
35. Raja R, Iqbal A, Hafiz Y, Willayet M, Bhat S, Rather M. Evaluation of bacteriological quality of ready-to-eat chicken products by total viable count method. J Adv Vet Res 2012;2:192-7.
36. Sooltan J, Mead G, Norris A. Incidence and growth potential of *Bacillus cereus* in poultrymeat products. Food Microbiol 1987;4:347-51.
37. Abostate MA, Zahran DA, El-Hifnawi H. Incidence of *Bacillus cereus* in some meat products and the effect of gamma; radiation on its toxin(s). Int J Agric Biol 2006;8:1-4.
38. Smith DP, Berrang ME, Feldner PW, Phillips RW, Meinersmann RJ. Detection of *Bacillus cereus* on selected retail chicken products. J Food Prot 2004;67:1770-3.
39. Rather MA, Aulakh RS, Gill JP, Rao TS, Hassan MN. Direct detection of *Bacillus cereus* and its enterotoxigenic genes in meat and meat products by polymerase chain reaction. J Adv Vet Res 2011;1:99-104.
40. Gueven K, Mutlu MB, Avci O. Incidence and characterization of *Bacillus cereus* in meat and meat products consumed in Turkey. J Food Saf 2006;26:30-40.
41. Luna VA, King DS, Gulledge J, Cannons AC, Amuso PT, Cattani J, *et al.* Susceptibility of *Bacillus anthracis*, *Bacillus cereus*, *Bacillus mycoides*, *Bacillus pseudomycoides* and *Bacillus thuringiensis* to 24 antimicrobials using Sensititre automated microbroth dilution and Etest agar gradient diffusion methods. J Antimicrob Chemother 2007;60:555-67.
42. Pirzada M, Malhi K, Memaon M, Leghari R, Leghari A, Habib F, *et al*. *In vitro* antimicrobial susceptibility profile of sub-clinical mastitis isolates from dairy goats. J Anim Health Prod 2016;4:72-7.
43. Agwa O, Uzoigwe C, Wokoma E. Incidence and antibiotic sensitivity of *Bacillus cereus* isolated from ready to eat foods sold in some markets in Portharcourt, Rivers State, Nigeria. Asian J Microbiol Biotechnol Environ Sci 2012;14:13-8.